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Isolation of Triterpenes and an Acetophenone Derivative with Antispasmodic Activity from *Euphorbia milli* Desmoul. ex Boiss. (Euphorbiaceae)

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SUMMARY. The aerial parts of *Euphorbia milli* Desmoul. ex Boiss. were studied. The results revealed the presence of β-amyrin acetate, α-amyrin acetate and 2,4-dihydroxy-6-methoxyacetophenone. The last two compounds have not been yet reported for this plant. Considering the similarity of acetophenone derivative with those of xanthoxyline, it was tested as antibacterial, antifungal and antispasmodic exhibiting only a moderate antispasmodic activity.

RESUMEN. "Aislamiento de Triterpenos y un Derivado de la Acetofenona con Actividad Antiespasmódica a partir de *Euphorbia milli* Desmoul. ex Boiss. (Euphorbiaceae)". Fueron estudiadas las partes aéreas de *Euphorbia milli* Desmoul. ex Boiss. Los resultados mostraron la presencia de acetato de β-amirina, acetato de α-amirina y 2,4-dihidroxi-6-metoxiacetofenona. Los dos últimos compuestos no fueron aún aislados en esta planta. La acetofenona sustituida, que es similar a xantoxilina, fue experimentada como antibacteriana, antifúngica y antiespasmódica, exhibiendo solamente una moderada actividad antiespasmódica.

INTRODUCTION

Several species of the genus *Euphorbia* (Euphorbiaceae) are widely distributed in Brazil. Some of these plants are used in folk medicine against different disorders and pharmacological reports have confirmed many medicinal properties [1,2]. We have previously investigated chemical and pharmacologically several plants of this family, including *Sebastiania schottiana* [3], *Phyllanthus sellowianus* [4,5], *Aleurites moluccana* [6], among others [7].

Euphorbia milli, known as "Coroa de Cristo" is used widely in the gardens as ornament. In some regions of the Brazil its latex is used to control the schistosomiasis [8]. Several classes of compounds have been described for *E. milli* Desmoul. ex Boiss., including terpenes [9], alkaloids [10] and phorbol derivatives [11].

In the present study, we have reported the isolation of two triterpenes and an acetophenone derivative. The biological activities of the last compound were evaluated in some experimental models.

KEY WORDS: Antispasmodic activity, *Euphorbia milli,* 2,4-dihydroxy-6-methoxyacetophenone, β and α-amyrin acetate .
PALABRAS CLAVE: Actividad antiespasmódica, *Euphorbia milli,* 2,4-dihidroxi-6-metoxiacetofenona, Acetato de β-amirina, Acetato de α-amirina.

MATERIAL & METHODS

Plant material

Aerial parts of *Euphorbia millii* were collected in December, 1993, in Florianópolis, SC, Brazil and classified by Professora Leila da Graça Amaral (Departamento de Botânica, UFSC, Florianópolis) and vouchers are deposited in Herbarium FLOR, UFSC, Florianópolis.

Isolation of constituents

The fresh material (2.7 kg) was minced and extracted exhaustively with hexane and ethyl acetate, respectively, for several days at room temperature. Ethyl acetate extract (3.5 g) was chromatographed on a silica gel column eluted with hexane- AcOEt gradient, yielding a white solid (10.0 mg) , identified as a mixture of triterpenes, α and β-amyrin acetate, and a white crystalline solid (294.0 mg), identified as 2,4-dihydroxy-6-methoxyacetophenone.

Analysis of triterpene mixture using HRGC indicated 45.5 % of β-amyrin acetate and 54.5 % of α-amyrin acetate **(1)**.

Spectral data: 2,4 - dihydroxy - 6 - methoxyacetophenone **(2)**:

IR (KBr, cm^{-1}): 3350 - 3050 (OH), 1637 (C=O), 1560 (C=C, Ar), 1359 (CH_3);

^{1}H-NMR ($CDCl_3$, 300 MHz, δ-values): 13.90 (s, 1H, OH); 6.00 (d, 1H, Ar, J=2.3 Hz); 5.90 (d, 1H, Ar, J= 2.3 Hz); 5.45 (s, 1H, OH); 3.80 (s, 3H, OCH_3); 2.60 (s, 3H, CH_3)

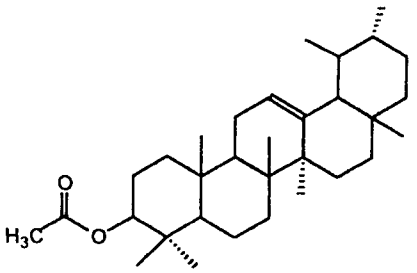
Biological assays

Antibacterial activity was evaluated by using diffusion method [12] against different pathogenic bacteria. Antifungal activity was analysed against mycelial and yeasts fungi by diffusion method in solid media [13]. Antispasmodic activity was tested against acetylcholine-induced contraction of the guinea pig isolated ileum using a kymograph, according previously described [14,15].

RESULTS AND DISCUSSION

Chromatographic procedures carried out with ethyl acetate extract obtained directly from aerial parts of *E. millii* yielded three compounds , as white solids. Less polar compound gave positive reaction with sulphuric-anisaldehyde suggesting to be a sterol or triterpene. The use of spectroscopic techniques (IR, NMR-^{1}H and ^{13}C) indicated a mixture of the known triterpenes β-amyrin acetate and α-amyrin acetate **(1)**. We have confirmed these structures by using HRGC in comparison with authentic samples. α-Amyrin acetate **(1)** is reported by the first time for this plant, as well as compound **(2)**, identified as 2,4-dihydroxy-6-methoxy acetophenone by direct comparison (IR, NMR-^{1}H, co-TLC) with an authentic sample.

Considering the similarity of **(2)** with xanthoxyline **(3)**, isolated from *Sebastiania schottiana,* which exhibits antispasmodic [3,15], antibacterial [16] and antifungal effects [17,18], we have evaluated these biological activities of **(2)**. Thus, against the pathogenic bacteria *S. aureus* and *E. coli,* by using diffusion method this compound was inactive at 500 μg/disk. In the same manner, it did not presented anti-



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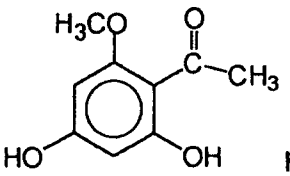

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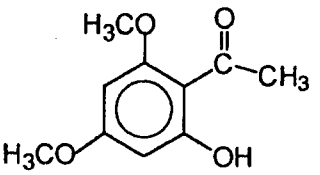

(3)

fungal activity against some mycelial and yeasts fungi, including *Candida albicans, Microsporum canis, Aspergillus flavus* and *Penicillium*. However, **(2)** exhibited a moderate antispasmodic effect against acetylcholine-induced contraction of guinea pig isolated ileum. The IC_{50} was approximately of 250 µM/ml, which is smaller than that of xanthoxyline (IC_{50} of 47 µM/ml) and 2,3-dimethoxyacetophenone (IC_{50} of 39 µM/ml)[15]. This fact confirm the suggestion that the methoxyl groups are important structural factors related with the antispasmodic activity of xanthoxyline derivatives [15].

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